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(71) Applicant *(for all designated States except US)*: **ST. GEORGE'S ENTERPRISES LIMITED** [GB/GB]; Cranmer Terrace, Tooting, London SW17 0RE (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **COATES, Anthony, Robert, Milnes** [GB/GB]; Dept. of Medical Microbiology, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE (GB).

(74) Agent: **THOMAS, Philip, J., D.**; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB).

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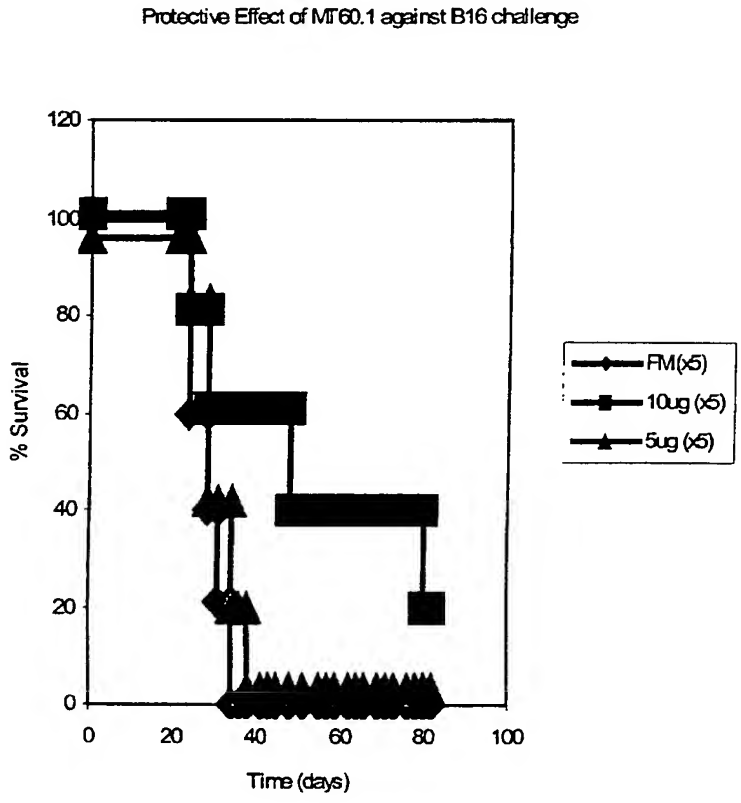

(57) Abstract: The invention relates to the use in the manufacture of a medicament for use in the treatment and/or prevention of a cancerous condition of an approx 60 kDa polypeptide (or its encoding nucleic acid molecules) or functionally equivalent molecules or fragments thereof from *Mycobacterium tuberculosis* or related prokaryotes.

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Biological materials and anti-cancer uses thereof

The present invention relates to the use of an approximately 60kDa polypeptide (or its encoding nucleic acid molecule) or functionally equivalent molecules or fragments thereof from *Mycobacterium tuberculosis* or related prokaryotes in the treatment of cancerous conditions.

Autoimmunity reflects the loss of tolerance to "self" resulting in inappropriate destruction of normal cells or tissue. In many conditions, autoantibodies are found, but may reflect an effect rather than cause of a disease. In some diseases however autoantibodies are the first, major, or only detectable abnormality. One class of molecules which is implicated in this respect are the chaperonins which are highly immunogenic. Chaperonins belong to a group of proteins called molecular chaperones which bind non-native proteins and assist them, in an ATP-dependent catalytic process, to fold into the correct three-dimensional form required for a functional protein.

Chaperonins are believed to stimulate the immune system at many levels simultaneously, including monocytes, macrophages, fibroblast-like cells, perhaps other types of cells, and T cells. The immune defences in mammals may be divided into the "innate" and "adaptive" defences. Those which are already in place, such as phagocytes, natural killer cells and complement are considered innate. On challenge, adaptive immunity is activated in the form of B and T lymphocytes. Chaperonins are known to act directly on the innate defence mechanisms, particularly on phagocytes. They also stimulate a powerful adaptive immune response, namely the production of antibody and the stimulation of T lymphocytes which in some cases may be protective. Notably they induce cytokine secretion which is

thought to be important for host defences. In some cases however it is believed that the presence of chaperonins may be damaging to the host.

Chaperonins' role in autoimmune disease is controversial. Although infection/immunity with chaperonin-containing organisms is universal, and healthy people have T cell responses to self-chaperonins, including the production of chaperonin-specific antibodies, classical autoimmune disease is quite uncommon. So the presence of immune reactions to chaperonins may be incidental and unimportant.

The theory of molecular mimicry however suggests the involvement of chaperonins in autoimmune disease and is based on the high level of amino acid sequence conservation between chaperonins of microbial and mammalian origin. The theory proposes that during infection with a wide range of microbes, chaperonin epitopes that are shared between microbes and mammals stimulate T lymphocytes. According to this theory a high level of chaperonin presentation of shared chaperonin epitopes breaks tolerance to self-chaperonins and autoimmune disease develops.

Chaperonins obtained from tumours have been found to result in necrotic effects on those tumours. It is suggested that this may be achieved through enhancing immunological recognition of tumour antigens although the mechanism of this is not known. It therefore appears that chaperonins induce protective adaptive immunity against bacterial infection and cancer.

Surprisingly it has now been found that another protein which is not known to be a heat shock protein or a chaperonin is able to affect the immunity of an individual and can be used for treating or preventing cancerous conditions.

This protein of unknown function has been identified in *Mycobacterium tuberculosis* and sequenced (Kong et al., 1993, Proc. Natl. Acad. Sci., 90, p2608-2612). Comparable proteins are known to exist in various other bacteria, including *M. bovis* and *Legionella*. It has been

named chaperonin 60.1 (cpn 60.1), but adoption of this nomenclature is simply based on its amino acid sequence identity to other chaperonins.

Chaperonin 60.2 (from the same source) exhibits 59.60% amino acid sequence identity and 65.6% nucleic acid sequence identity to cpn 60.1 using the alignment methods described hereinafter. Cpn 60.2 in common with cpn 60.1 does not have confirmed chaperonin properties. Chaperonins are believed to function by the formation of 2 ring heptamers (composed of approximately 60kDa monomers) which face one another and are capped by a ring heptamer composed of approximately 10kDa monomers (formed by cpn 10s). Assisted folding is achieved once the target protein has entered into the central core, whereafter it is released. Thus the formation of the heptamers appears to be essential to the presently understood functionality of chaperonins. However, unlike cpn 60s from other species, it has not been found possible to produce heptamers of *M. tuberculosis* cpn 60.1. Furthermore, unlike the GroE chaperonin folding machinery, neither the cpn 60.1 gene nor the cpn 60.2 gene is in the same operon as the chaperonin cpn 10 gene and thus transcription of the components which are necessary for the formation of a chaperonin complex is not under the same control mechanisms.

It has also been observed that the cpn 60.1 protein has a unique histidine-rich sequence at the C-terminus unlike cpn 60s from other species which usually have a sequence rich in glycine and methionine. The 60.2 protein is a known heat shock protein and has very high homology to related heat shock proteins in other species, e.g. 95% identity to the same protein from M. *leprae*. As mentioned above, cpn 60.2 is situated distant to cpn 60.1 on the genome of *M. tuberculosis* and is under distinct transcriptional control. Cpn 10 which is a chaperonin and a heat shock protein is also under discrete transcriptional control to the molecules cpn 60.1 and 60.2. As a consequence there is no evidence to suggest that cpn 60.1 is either a heat

shock protein or a chaperonin. These facts strongly suggest different functional roles for the cpn 60 proteins in *M. tuberculosis*.

The invention therefore provides molecules such as cpn 60.1 in the manufacture of a pharmaceutical composition for use in treating or preventing various cancerous conditions. Therapeutic and/or prophylactic applications may be achieved using nucleic acid molecules or peptides/proteins, as will be described in more detail hereinafter.

In a first aspect the present invention provides use of a nucleic acid molecule comprising

(i) the nucleotide sequence of Figure 1, or

(ii) a sequence which has more than 66%, e.g. 70 or 75%, preferably more than 80%, e.g. more than 90 or 95% identity to sequence (i) (according to the test described hereinafter) or a sequence which hybridizes to sequence (i) under conditions of 2 x SSC, 65°C (wherein SCC = 0.15M NaCl, 0.015M sodium citrate, pH 7.2) which encodes a functionally equivalent protein to the sequence encoded by the nucleotide sequence of Figure 1, or (iii) a fragment of sequence (i) or (ii) encoding a functionally equivalent protein fragment; and a pharmaceutically acceptable excipient, diluent or carrier in the manufacture of a medicament for use in treating and/or preventing a cancerous condition.

As mentioned above, therapeutic and/or prophylactic effects may be achieved using nucleic acid molecules or peptide/protein molecules. Thus in a further aspect the present invention provides use of a pharmaceutical composition comprising a polypeptide comprising

(i) the amino acid sequence of Figure 1, or

(ii) a sequence which has more than 60%, e.g. 65 or

70%, preferably more than 80%, e.g. more than 90 or 95% homology to sequence (i) (according to the test described hereinafter) which provides a functionally equivalent protein, or

(iii) a functionally equivalent fragment of sequence (i) or (ii); and a pharmaceutically acceptable excipient, diluent or carrier in the manufacture of a medicament for use in treating and/or preventing a cancerous condition.

"Nucleic acid molecules" according to the invention may be single or double stranded DNA, cDNA or RNA, preferably DNA. Derivatives of nucleotide sequences capable of encoding functionally-equivalent polypeptides may be obtained by using conventional methods well known in the art.

Nucleic acid molecules for use in the invention may consist only of sequences derived from Figure 1 (or related functionally equivalent sequences), or may comprise additional sequences, such as structural or functional sequences, e.g. sequences which control transcription and/or expression (particularly in mammalian cells), or sequences which comprise the sequence for an additional protein moiety which may form a fusion protein which may have specific properties e.g. act as a secretary signal. Thus for example the sequence may be in the form of a vector containing the nucleic acid molecules described herein. Suitable vectors include plasmids and viruses.

"Polypeptides" as referred to herein includes both full-length protein and shorter length peptide sequences, e.g. protein fragments as described herein. Such polypeptides may be prepared by any convenient means, e.g. by isolation from the source prokaryote or by recombinant means by expression of the appropriate nucleic acid molecule in a host cell operatively linked to an expression control sequence, or a recombinant DNA cloning

vehicle or vector containing such a recombinant DNA molecule or by chemical or biochemical synthesis (ex vivo).

"Sequence identity" as referred to herein in connection with nucleotide sequences refers to the value obtained when assessed using ClustalW (Thompson et al., 1994, Nucl. Acids Res., 22, p4673-4680) with the following parameters:

Pairwise alignment parameters - Method: accurate,

Matrix: IUB, Gap open penalty: 15.00, Gap extension penalty: 6.66;

Multiple alignment parameters - Matrix: IUB, Gap open penalty: 15.00, % identity for delay: 30, Negative matrix: no, Gap extension penalty: 6.66, DNA transitions weighting: 0.5.

In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson et al., 1994, supra) with the following parameters: Pairwise alignment parameters - Method: accurate,

Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10;

Multiple alignment parameters - Matrix: PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on, Hydrophilic residues: GPSNDQEKR. Sequence identity at a particular residue is intended to include identical residues which have simply been derivatized.

"Functionally equivalent" proteins or protein fragments refers to proteins or fragments related to, or derived from the amino acid sequence of Figure 1, where the amino acid sequence has been modified by single or multiple amino acid (e.g. at 1 to 50, e.g. 10 to 30, preferably 1 to 5 bases) substitution, addition and/or deletion but which nonetheless retains functional activity, e.g. suppresses ovalbumin-induced eosinophilia, for

example reducing eosinophil numbers to the extent of more than 10 %, e.g. more than 25%, particularly preferably more than 50% and/or an increase in the production of specific cytokines such as interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-10, IL-12, IL-12 receptor, tumour necrosis factor a (TNFα), interferon-γ and granulocyte-macrophage-colony stimulating factor (GM-CSF) e.g. a more than 10 fold, preferably more than 100 fold increase over normal levels and/or stimulation of Thl responses. Cytokine assays such as ELISA are within the common knowledge of skilled persons.

Within the meaning of "addition" variants are included amino and/or carboxyl terminal fusion proteins or polypeptides, comprising an additional protein or polypeptide fused to the polypeptide sequence.

Particularly preferred are naturally occurring equivalents such as biological variations, e.g. allelic, geographical or allotypic variants and derivatives prepared using known techniques. For example, functionally-equivalent proteins or fragments may be prepared either by chemical peptide synthesis or in recombinant form using the known techniques of site-directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

The invention is particularly directed to homologues and related molecules from different prokaryotes, e.g. from bacterial genera, species or strains, particularly from the genus *Mycobacterium, e.g.* homologues from the *Mycobacterium tuberculosis* complex which includes *M. tuberculosis, M. bovis* and *M. africanum.* Such sequences may themselves be modified, particularly derivatized providing they still retain functionality.

Derivatives of the proteins may be prepared by post-synthesis/isolation modification or by modification during synthesis, e.g. using modified residues or expression of modified nucleic acid molecules, where appropriate.

Functionally-equivalent fragments according to the invention may be made by truncation, e.g. by removal of a peptide from the N and/or C-terminal ends or by selection of an appropriate active domain region, e.g. an epitopic region which retains its functionality. Such fragments may be derived from the sequence of Figure 1 or may be derived from a functionally equivalent protein to that disclosed in Figure 1.

It will be appreciated that where functional fragments are selected they may not exhibit all functions attributed to the source molecules. Thus functionally equivalent proteins or fragments refers to retention of relevant functional properties such that the fragment retains utility according to the invention, e.g. reduces eosinophilia, increases the production of specific cytokines and/or stimulates the Th1 immune response, as mentioned above.

Preferably the fragments are between 6 and 400 residues in length, e.g. 6 to 100 or 15 to 100 residues, preferably 6 to 30, 10 to 25, 15 to 50 or 15 to 30 residues. Particularly preferred fragments are those derived from or consisting of residues:

1-8	MSKLIEYD, (8)
14-21	AMEVGMDK, (8)
40-48	AKAFGGPTV, (9)
64-71	PFEDLGAQ, (8)
96-105	QALIKGGLRL, (11)
110-129	VNPIALGVGIGKAADAVSEA, (20)
132-143	ASATPVSGKTGI, (12)
144-155	AQVATVSSRDEQ, (12)
160-175	VGEAMSKVGHDGVVSV, (16)
179-200	STLGTELEFTEGIGFDKGFLSA, (22)
195-219	KGFLSAYFVTDFDNQQAVLEDALIL, (25)
206-219	FDNQQAVLEDALIL, (14)
221-229	HQDKISSLP, (9)

264-271	AIRKTLKA, (8)
276-293	GPYFGDRRKAFLEDLAVV, (18)
299-314	VNPDAGMVLREVGLEV, (16)
315-326	LGSARRVVVSKD (12)
327-342	DTVIVDGGGTAEAVAN, (16)
343-353	RAKHLRAEIDK, (11)
379-391	VGAATETALKERK (13)
392-400	ESVEDAVAA, (9)
411-433	PGGGASLIHQARKALTELRASLT, (23)
434-449	GPEVLGVDVFSEALAA, (16)
450-463	PLFWIAANAGLDGS, (14)
464-471	VVVNKVSE, (8)
480-494	VNTLSYGDLAADGVI, (15)
501-526	RSAVLNASSVARMVLTTETVVVDKPA, (15)
526-539	KAEDHDHHHGHAH. (14)

Functionally equivalent nucleic acid sequences/fragments compared to the sequence recited in Figure 1 are also used in compositions of the invention. These sequences are defined with reference to the functionally equivalent protein/peptides (as defined above) which they encode.

"Hybridisation" as used herein refers to those sequences which bind under non-stringent conditions (6 x SSC/50% formamide at room temperature) and washed under conditions of high stringency e.g. 2 x SSC, 65°C (where SSC = 0.15M NaCl, 0.015M sodium citrate, pH 7.2).

"Pharmaceutically acceptable" as referred to herein refers to ingredients that are compatible with other ingredients of the compositions as well as physiologically acceptable to the recipient.

Pharmaceutical compositions according to the invention may be formulated in conventional manner using readily available ingredients. Thus, the active ingredient (ie. the nucleic acid molecule or protein/peptide),

may be incorporated, optionally together with other active substances, with one or more conventional carriers, diluents and/or excipients, to produce conventional galenic preparations such as tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments, soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders, and the like.

As mentioned above, compositions may additionally comprise molecules which assist or augment the action of the nucleic acid molecules or polypeptides described hereinbefore, e.g. thalidomide (and analogues thereof), low dose cyclophosphamide, LPS, cytokines, chemokines, CpG oligodeoxynucleotides and other immunomodulators and/or anti-inflammatory agents such as cytokine antagonists or glucocorticosteroids.

Thus for example, the compositions may be used together with active ingredients for specific immunotherapies e.g. in cancer vaccines. Appropriate immunotherapy treatment/vaccine preparations which may include nucleic acid molecules/polypeptides as described herein include subunit vaccines or treatments based on tumour specific antigens or tumour associated antigens or antibody, anti-idiotype antibody or whole cell preparations for vaccination or therapy. When used in therapy or vaccination the nucleic acid molecules or polypeptides described herein may provide (or encode) an antigen resulting in a specific immune response directed to that antigen and/or may result in a general and nonspecific immune response. In the latter case in which compositions containing other active ingredients are used, the nucleic acid molecules/polypeptides described herein act as adjuvants and may be used for this purpose.

Preventative or therapeutic preparations may be formulated to include one or more suitable adjuvants, e.g. Incomplete Freund's Adjuvant, BCG, Montanide, aluminium hydroxide, saponin, quil A, or more purified

forms thereof, muramyl dipeptide, mineral or vegetable oils, Novasome or non-ionic block co-polymers or DEAE dextran, in the presence of one or more pharmaceutically acceptable carriers or diluents. Suitable carriers include liquid media such as saline solution.

Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, aglinates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, water, water/ethanol, water/glycol, water/polyethylene glycol, propylene glycol, methyl cellulose, methylhydroxybenzoates, propyl hydroxybenzoates, talc, magnesium stearate, mineral oil or fatty substances such as hard fat or suitable mixtures thereof. The compositions may additionally include lubricating agents, wetting agents, emulsifying agents, suspending agents, preserving agents, sweetening agents, flavouring agents, and the like. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

Compositions may be in an appropriate dosage form, for example as an emulsion or in liposomes, niosomes, microspheres, nanoparticles or the like.

If required, the compositions may also contain targeting moieties attached to the active ingredient, e.g. a ligand which binds specifically and selectively to an endogenous receptor to allow targeting to a particular cell type or location, such as targeting to cancer cells.

The above described compositions have utility in the treatment or prophylaxis of cancerous conditions.

Alternatively viewed, the present invention provides a method of treating or preventing cancerous conditions in a patient wherein said patient is administered a pharmaceutical composition as described hereinbefore.

Furthermore, the present invention provides the use of a nucleic acid molecule comprising

(i) the nucleotide sequence of Figure 1, or

(ii) a sequence which has more than 66%, e.g. 70 or 75%, preferably more than 80%, e.g. more than 90 or 95% identity to sequence (i) (according to the test described hereinbefore) or a sequence which hybridizes to sequence (i) under conditions of 2 x SSC, 65°C (wherein SCC = 0.15M NaCl, 0.015M sodium citrate, pH 7.2) which encodes a functionally equivalent protein to the sequence encoded by the nucleotide sequence of Figure 1, or (iii) a fragment of sequence (i) or (ii) encoding a functionally equivalent protein fragment; or

a polypeptide comprising

(i) the amino acid sequence of Figure 1, or

(ii) a sequence which has more than 60%, e.g. 65 or 70%, preferably more than 80%, e.g. more than 90 or 95% homology to sequence (i) (according to the test described hereinbefore) which provides a functionally equivalent protein, or

(iii) a functionally equivalent fragment of sequence (i) or (ii);

in the preparation of a medicament for treating or preventing a cancerous condition.

As defined herein "treatment" refers to reducing, alleviating or eliminating one or more symptoms of the condition which is being treated, relative to the symptoms prior to treatment. For example, symptoms which may be affected include eosinophilia, decreased secretion of particular cytokines, a Th2-biased immune response, tumour size (e.g. by halting proliferation, causing differentiation, enhancing or inducing antitumour immune responses or causing some cell death), allergic response, presence of autoantibodies, etc which are treated to achieve the effects particularly as

defined in respect of the functional properties of functionally equivalent polypeptides.

"Prevention" of a condition refers to delaying or preventing the onset of a condition or reducing its severity, as assessed by the appearance or extent of one or more symptoms of said condition.

In particular, cancerous conditions which may be treated include malignant and pre-malignant or benign tumours and include carcinomas, sarcomas, glioma, melanoma and Hodgkin's disease, including cancers of the bladder, kidney, pancreas, brain, head and neck, breast, gut, prostate, lung and ovary and leukaemias and lymphomas.

Patients which may be treated include, but are not limited to mammals, particularly primates, domestic animals and livestock. Thus preferred animals for treatment include mice, rats, guinea pigs, cats, dogs, pigs, goats, sheep, horses and particularly preferably, humans.

As mentioned previously, either nucleic acid molecules or polypeptides may be used in the methods of the invention. In instances in which nucleic acid molecules are employed, these are conveniently applied in a form to allow their expression within the patient, thus providing a form of gene therapy. Thus the pharmaceutical compositions described herein containing a nucleic acid molecule may be used in methods of gene therapy.

Thus for example the nucleic acid molecules may be provided in a liposome, micelle or other convenient carrying vehicle which may comprise targeting moieties to allow its targeting to cells of interest.

Alternatively the molecules may be packaged in other, "vehicles" such as viruses, plasmids or cells (particularly transfected species-matched cells) which are all well known in the art for this purpose which allow expression of the resident molecule.

Appropriate techniques for transfection are well known and include electroporation, microinjection, lipofection, adsorption, viral transfection and protoplast fusion.

Administration of compositions of the invention may take place by any of the conventional routes, e.g. by inhalation, nasally, orally, rectally or parenterally, such as by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Treatment or prophylaxis by topical application of a composition, e.g. an ointment, to the skin is also possible. Optionally administration may be performed at intervals, e.g. 2 or more applications, e.g. 2-4 applications at hourly, daily, weekly or monthly intervals, e.g. several times a day, or every 3-5 days, or at fortnightly, monthly or quarterly intervals.

It has been observed in work conducted on the related molecule cpn 60.2 that the route of administration may affect the immune response which is generated. For example when Mtcpn 60.2 is administered intranasally, a Th2 to Thl shift is stimulated although the reverse effect is observed when administered intraperitoneally. Thus, the route of administration should take into account the disorder to be treated/prevented and thus for example in treating cancerous conditions or autoimmune disorders, intraperitoneal administration may be appropriate whereas treatment or prevention of particularly allergic disorders may be for example by intranasal administration.

In prophylactic methods of the invention, administration (conveniently orally or by inhalation or subcutaneous or intramuscular injection) is preferably performed at more lengthy intervals, e.g. intervals of 2-12 weeks. For therapeutic purposes, administration (conveniently orally or by inhalation or intravenous injection) is performed 1-4 times in a single day or over 2 days.

The active ingredient in composition of the invention may comprise from about 0.01% to about 99% by weight of the formulation, preferably from about 0.1 to about 50%, for example 10%. The compositions are preferably formulated in a unit dosage form, e.g. with each dosage containing from about 0.0lmg to about 1g of the active ingredient, e.g. 0.05mg to 0.5g, for a human, e.g. 1-100mg.

The precise dosage of the active compound to be administered and the length of the course of treatment will, of course, depend on a number of factors including for example, the age and weight of the patient, the specific condition requiring treatment and its severity, and the route of administration. Generally however, an effective dose may lie in the range of from about 0.lμg/kg to about 14mg/kg, preferably 0.1 to 1mg/kg, e.g. from about 1mg to 1g of polypeptide per day, depending on the animal to be treated and the dosage form, taken as a single dose. Thus for example, an appropriate daily dose for an adult may be from 7μg to lg, e.g. 10mg to lg per day, e.g. 25 to 500mg of the polypeptide per day.

Similar or lower dosages may be used when using nucleic acid molecules described herein, e.g. from about 0.2ng/kg to about 2.5mg/kg (e.g. from about 0.2ng/kg to about 2ng/kg or about 1.5ng/kg to about 2.5mg/kg) such as about 14ng to about 175mg for an adult. However, where the nucleic acid molecules are packaged in cells or vectors proportionally higher or lower amounts may be required depending on the extent of non-cpn encoding DNA and sequences which influence the level of expression, e.g. 5 or 10-fold larger amounts, e.g. nucleic acid molecules described herein packaged in a vector may be used at about 1.0ng/kg to about 12.5mg/kg.

Definitions

"AUTOIMMUNE DISEASE". This term intended to cover those cases where it can be shown that the autoimmune process contributes to the pathogenesis of a disease. Such diseases are typically associated with a Thelper lymphocyte-1 (Th-1) type immune response.

"ADJUVANT". This term is intended to cover any substance which, when incorporated into or administered simultaneously with antigen, potentiates the immune response.

"MT60.1", "Mtcpn60.1" "cpn 60.1", and "60.1" are used interchangeably throughout the specificaiton to refer to the amino acid sequence shown in Figure 1.

The invention will now be described in more detail by way of the following non-limiting Examples in which:-

Figure 1 shows the nucleotide and amino acid sequence of cpn 60.1 from *M. tuberculosis;*

Figure 2 shows the therapeutic effect of 60.1 on Melanoma.

Figure 3 shows the therapeutic effect of 60.1 when used in combination with allogeneic cells.

Materials

Expression and purification of chaperonin 60 proteins M. tuberculosis cpn 60.1 was prepared using conventional chromatography as described below.

Purification of recombinant cpn 60.1:

The pellet of a recombinant *E.coli* BL21 (DE3) culture containing a plasmid encoding for *M. tuberculosis* cpn 60.1 (ITPG-induced) was suspended in detergent containing buffer and disrupted by sonication. BL21 contained recombinant plasmid pWO2-1, a plasmid which contains the cpn60.1 gene (Kong et al., 1993, supra) cloned into the expression vector pET22b (Guerroro et al., 1999, Eur. J. Biochem., 259, p789-794). The inclusion bodies were solubilized in 8M urea followed by refolding by gel filtration. The protein was purified by chelating his-tag affinity chromatography. The final buffer was changed to 10mM ammonium bicarbonate by dialysis. Cpn 60.1 was aliquotted and lyophilized.

Great care was taken to check each batch of protein for LPS contamination using the Limulus assay (Tabona et al., 1998, J. Immunol., 161, p1414-1421). If LPS contamination was detected it was removed on a polymyxin B affinity column and levels of LPS re-assayed. Recombinant, LPS-low, 60.1 protein was further purified on a Reactive Red column to remove contaminating proteins and peptides (Tabona et al., 1998, supra).

Example 1 - Anti-Cancer Use

Effect of 60.1 treatment on survival in mouse model of melanoma.

The use of 60.1 was tested in a mouse model of melanoma. This is a variant of the classic B16 melanoma model, in which allogeneic melanoma cells are administered as a vaccine, followed by syngeneic B16 challenge.

Method

1. 8x5 C57/b16 mice ($H2^b$) were challenged sub-cutaneously, in the right flank, with B16-F10 clone G1 (1.10^4) cells per 0.2ml dose. Clone G1 was made by limiting dilution at Onyvax Ltd.

2. 1.2mls of each vaccine was made up to ensure adequate volume for 0.2mls in 5 mice. The vaccines were made up with 0.9% injectible saline (B. Braun Ltd., Sheffield, UK). Allogeneic melanoma was K1735 clone G10; cloned by Onyvax Ltd. The cells were used as a frozen vaccine made by resuspending cells in freezing medium, comprising RPM1-1640, 10% fetal calf serum and 10% DMSO, and then freezing cells at -70°C. This same freezing medium was also used as a control. 60.1 was stored at -20°C in a stock of 2.25mg/ml.

	per ml	per µl	in 1.2mls
5µg	22.2	0.022	26.64
10µg	44.4	0.044	53.28

3. Animals were vaccinated in the contralateral flank to that of the tumour challenge on days 3, 7, 10, 13 & 16 with 0.2mls of the following vaccines ($5x10^6$ per ml).

Group	Challenge	3	7	10	13	16
1	B16	K	K	K	K	K
2	B16	5μg/K	5μg/K	5μg/K	5μg/K	5μg/K
3	B16	5μg	5μg	5μg	5μg	5μg
4	B16	10μg/K	10μg/K	10μg/K	10μg/K	10μg/K
5	B16	10μg	10μg	10μg	10μg	10μg
6	B16	10μg/K	10μg/K	K	K	K
7	B16	10μg	10μg	FM	FM	FM
8	B16	FM	FM	FM	FM	FM

Key: K=K1735; 5 or 10μg= MT60.1; FM=freezing medium

4. Tumour size was monitored every two days and animals bearing tumours greater than 15mm in either axis killed by cervical dislocation.

Results

The effect of MT60.1 on its own as a therapy is shown in Fig 2. Clearly, the molecule has a therapeutic effect. This is further supported by the fact that administration of a lower dose lessens the effect, suggesting a dose response.

When use in combination with allogeneic cells, MT60.1 was also effective (Fig 3) although interestingly, the protective effect is only manifest at the lower dose. This may be explained by the observation that allogeneic cells alone gave excellent protection. Hence the addition of a second therapeutic agent will only be required at a lower does since the immune system has already been activated by the allogeneic cells.

The *in vitro* effects of cpn 60.1 on the production of IL-1β, IL-6, IL-8, IL-10, IL-12, TNFα and GM-CSF in human PBMCs can be determined using 2-site ELISA as described by Tabona et al., 1998, supra.

Claims

1. Use, in the manufacture of a medicament for use in the treatment and/or prevention of a cancerous condition, of a nucleic acid molecule comprising:

(i) the nucleotide sequence of Figure 1, or

(ii) a sequence which has more than 66% identity to sequence (i), or a sequence which hybridizes to sequence (i) under conditions of 2 x SSC, 65°C (wherein SCC = 0.15M NaCl, 0.15M sodium citrate, pH 7.2) which encodes a functionally equivalent protein to the sequence encoded by the nucleotide sequence of Figure 1, or

(iii) a fragment of sequence (i) or (ii) encoding a functionally equivalent protein fragment.

2. Use, in the manufacture of a medicament for use in the treatment and/or prevention of a cancerous condition, of a polypeptide comprising:

(i) the amino acid sequence of Figure 1, or

(ii) a sequence which has more than 60 homology to sequence (i) which provides a functionally equivalent protein, or

(iii) a functionally equivalent fragment of sequence (i) or (ii).

3. Use as claimed in Claim 2 wherein the fragments are from 6 to 400 residues in length.

4. Use as claimed in Claim 3 wherein the fragmenets are from 6 to 100 residues in length.

5. Use as claimed in Claim 4 wherein the fragments are derived from or consisting of at least one of the following residues:

1-8	MSKLIEYD, (8)
14-21	AMEVGMDK, (8)
40-48	AKAFGGPTV, (9)
64-71	PFEDLGAQ, (8)
96-105	QALIKGGLRL, (11)
110-129	VNPIALGVGIGKAADAVSEA, (20)
132-143	ASATPVSGKTGI, (12)
144-155	AQVATVSSRDEQ, (12)
160-175	VGEAMSKVGHDGVVSV, (16)
179-200	STLGTELEFTEGIGFDKGFLSA, (22)
195-219	KGFLSAYFVTDFDNQQAVLEDALIL, (25)
206-219	FDNQQAVLEDALIL, (14)
221-229	HQDKISSLP, (9)
264-271	AIRKTLKA, (8)
276-293	GPYFGDRRKAFLEDLAVV, (18)
299-314	VNPDAGMVLREVGLEV, (16)
315-326	LGSARRVVVSKD (12)
327-342	DTVIVDGGGTAEAVAN, (16)
343-353	RAKHLRAEIDK, (11)
379-391	VGAATETALKERK (13)
392-400	ESVEDAVAA, (9)
411-433	PGGGASLIHQARKALTELRASLT, (23)
434-449	GPEVLGVDVFSEALAA, (16)
450-463	PLFWIAANAGLDGS, (14)
464-471	VVVNKVSE, (8)
480-494	VNTLSYGDLAADGVI, (15)
501-526	RSAVLNASSVARMVLTTETVVVDKPA, (15)

526-539 KAEDHDHHHGHAH. (14)

6. Use as claimed in Claim 1 wherein the nucleic acid molecule encodes a fragment as defined in any one of Claims 2 to 5.

7. Use as claimed in any preceding claim in combination with at least one active ingredient for assisting or augmenting the action of the nucleic acid molecules or polypeptides.

8. Use as claimed in Claim 7 wherein the active ingredient is selected from at least one of subunit: vaccines, treatments based on tumour specific antigens or antibody, or tumour associated antigens or antibody, anti-idiotype antibody, or whole cell preparations for vaccination or therapy.

9. A method for treating or preventing a cancerous condition comprising administering to a patient a therapeutically effective amount of a medicament as defined in any preceding claim.

10. A use or method as claimed in any preceding claim wherein the cancerous condition is selected from at least one of:

carcinomas, sarcomas, glioma, melanoma and Hodgkin's disease, including cancers of the bladder, kidney, pancreas, brain, head and neck, breast, gut, prostate, lung and ovary and leukaemias and lymphomas.

11. Use as claimed in any one of Claims 1 to 8 or 10 wherein the cancerous condition is a cancerous condition of a human.

12. A method as claimed in Claim 9 or 10 wherein the patient is a human.

Figure 1

```
   1  ATGAGCAAGCTGATCGAATACGACGAAACCGCGCGTCGCGCCATGGAGGTCGGCATGGAC   60
      M  S  K  L  I  E  Y  D  E  T  A  R  R  A  M  E  V  G  M  D

  61  AAGCTGGCCGACACCGTGCGGGTGACGCTGGGGCCGCGCGGCCGGCATGTGGTGCTGGCC  120
      K  L  A  D  T  V  R  V  T  L  G  P  R  G  R  H  V  V  L  A

 121  AAGGCGTTTGGCGGACCCACGGTTACCAACGACGGCGTCACGGTGGCACGTGAGATCGAG  180
      K  A  F  G  G  P  T  V  T  N  D  G  V  T  V  A  R  E  I  E

 181  CTGGAAGATCCGTTTGAAGACTTGGGCGCCCAGCTGGTGAAGTCGGTGGCCACCAAGACC  240
      L  E  D  P  F  E  D  L  G  A  Q  L  V  K  S  V  A  T  K  T

 241  AACGATGTGGCCGGTGACGGCACCACCACCGCAACCATCTTGGCGCAGGCACTGATCAAG  300
      N  D  V  A  G  D  G  T  T  T  A  T  I  L  A  Q  A  L  I  K

 301  GGCGGCCTGAGGCTAGTGGCCGCCGGCGTCAACCCGATCGCGCTCGGCGTGGGAATCGGC  360
      G  G  L  R  L  V  A  A  G  V  N  P  I  A  L  G  V  G  I  G

 361  AAGGCCGCCGACGCGGTATCCGAGGCGCTGCTGGCATCGGCCACGCCGGTGTCCGGCAAG  420
      K  A  A  D  A  V  S  E  A  L  L  A  S  A  T  P  V  S  G  K

 421  ACCGGCATCGCGCAGGTGGCGACGGTGTCCTCGCGCGACGAGCAGATCGGTGACCTGGTT  480
      T  G  I  A  Q  V  A  T  V  S  S  R  D  E  Q  I  G  D  L  V

 481  GGCGAAGCGATGAGCAAGGTCGGCCACGACGGCGTGGTCAGCGTCGAAGAATCCTCGACG  540
      G  E  A  M  S  K  V  G  H  D  G  V  V  S  V  E  E  S  S  T

 541  CTGGGCACCGAGTTGGAGTTCACCGAGGGTATCGGCTTCGACAAGGGCTTCTTGTCGGCA  600
      L  G  T  E  L  E  F  T  E  G  I  G  F  D  K  G  F  L  S  A

 601  TACTTCGTTACCGACTTCGATAACCAGCAGGCGGTGCTCGAGGACGCGTTGATCCTGCTG  660
      Y  F  V  T  D  F  D  N  Q  Q  A  V  L  E  D  A  L  I  L  L

 661  CACCAAGACAAGATCAGCTCGCTTCCCGATCTGTTGCCATTGCTGGAAAAGGTTGCAGGA  720
      H  Q  D  K  I  S  S  L  P  D  L  L  P  L  L  E  K  V  A  G

 721  ACGGGTAAGCCACTACTGATCGTGGCTGAAGACGTGGAGGGCGAAGCGTTGGCGACGCTG  780
      T  G  K  P  L  L  I  V  A  E  D  V  E  G  E  A  L  A  T  L

 781  GTCGTCAACGCGATTCGCAAGACGTTGAAAGCGGTCGCGGTCAAGGGGCCGTACTTCGGT  840
      V  V  N  A  I  R  K  T  L  K  A  V  A  V  K  G  P  Y  F  G

 841  GACCGCCGTAAGGCGTTCCTTGAGGACCTGGCGGTGGTGACGGGTGGCCAGGTGGTCAAC  900
      D  R  R  K  A  F  L  E  D  L  A  V  V  T  G  G  Q  V  V  N

 901  CCCGACGCCGGCATGGTGCTGCGCGAGGTGGGCTTGGAGGTGCTGGGCTCGGCCCGACGC  960
      P  D  A  G  M  V  L  R  E  V  G  L  E  V  L  G  S  A  R  R

 961  GTGGTGGTCAGCAAGGACGACACGGTCATTGTCGACGGCGGCGGCACCGCAGAAGCGGTG 1020
      V  V  V  S  K  D  D  T  V  I  V  D  G  G  G  T  A  E  A  V

1021  GCCAACCGGGCGAAGCACTTGCGTGCCGAGATCGACAAGAGCGATTCGGATTGGGATCGG 1080
      A  N  R  A  K  H  L  R  A  E  I  D  K  S  D  S  D  W  D  R

1081  GAAAAGCTTGGCGAGCGGCTGGCCAAACTGGCCGGCGGGGTTGCTGTCATCAAGGTGGGT 1140
      E  K  L  G  E  R  L  A  K  L  A  G  G  V  A  V  I  K  V  G

1141  GCCGCCACCGAGACCGCACTCAAGGAGCGCAAGGAAAGCGTCGAGGATGCGGTCGCGGCC 1200
      A  A  T  E  T  A  L  K  E  R  K  E  S  V  E  D  A  V  A  A

1201  GCCAAGGCCGCGGTCGAGGAGGGCATCGTCCCTGGTGGGGGAGCCTCGCTCATCCACCAG 1260
      A  K  A  A  V  E  E  G  I  V  P  G  G  G  A  S  L  I  H  Q
```

Figure 1 (cont'd)

```
1261  GCCCGCAAGGCGCTGACCGAACTGCGTGCGTCGCTGACCGGTGACGAGGTCCTCGGTGTC  1320
      A  R  K  A  L  T  E  L  R  A  S  L  T  G  D  E  V  L  G  V

1321  GACGTGTTCTCCGAAGCCCTTGCCGCGCCGTTGTTCTGGATCGCCGCCAACGCTGGCTTG  1380
      D  V  F  S  E  A  L  A  A  P  L  F  W  I  A  A  N  A  G  L

1381  GACGGCTCGGTGGTGGTCAACAAGGTCAGCGAGCTACCCGCCGGGCATGGGCTGAACGTG  1440
      D  G  S  V  V  V  N  K  V  S  E  L  P  A  G  H  G  L  N  V

1441  AACACCCTGAGCTATGGTGACTTGGCCGCTGACGGCGTCATCGACCCGGTCAAGGTGACT  1500
      N  T  L  S  Y  G  D  L  A  A  D  G  V  I  D  P  V  K  V  T

1501  AGGTCGGCGGTGTTGAACGCGTCATCGGTTGCCCGGATGGTACTCACCACCGAGACGGTC  1560
      R  S  A  V  L  N  A  S  S  V  A  R  M  V  L  T  T  E  T  V

1561  GTGGTCGACAAGCCGGCCAAGGCAGAAGATCACGACCATCACCACGGGCACGCGCACTGA  1620
      V  V  D  K  P  A  K  A  E  D  H  D  H  H  H  G  H  A  H  *
```

Figure 2

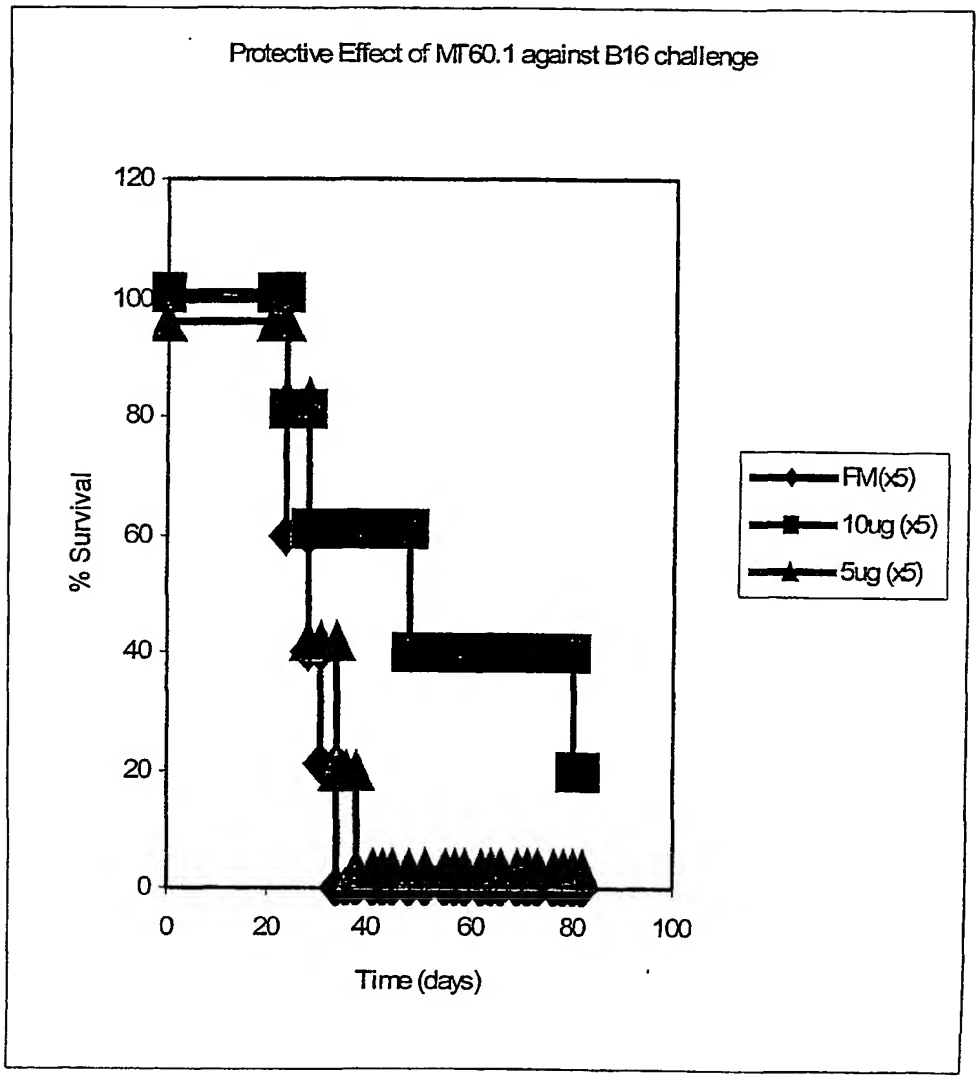
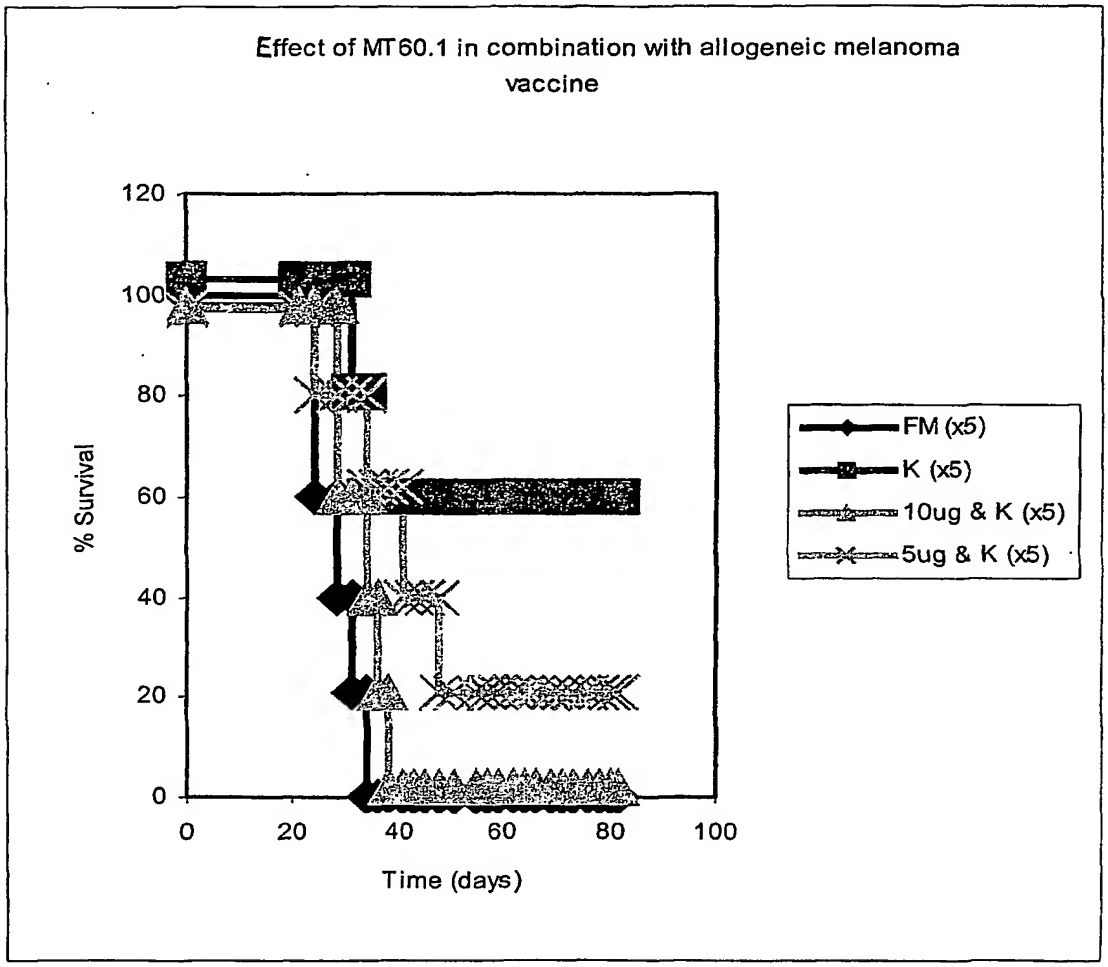

Protective Effect of MT60.1 against B16 challenge
% Survival
120
100
80
60
40
20
0
0
20
40
60
80
100
Time (days)
FM(x5)
10ug (x5)
5ug (x5)

Figure 3


Effect of MT60.1 in combination with allogeneic melanoma vaccine
% Survival
120
100
80
60
40
20
0
0
20
40
60
80
100
Time (days)
FM (x5)
K (x5)
10ug & K (x5)
5ug & K (x5)